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AbSieve:

Combined strength of Phage and Yeast Display for finding better drug candidates

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Introduction

High expression yields and good molecule stability are key features of drug candidates that should be addressed as early in the discovery process as possible. Therefore, a yeast display step was implemented into our antibody discovery technology platform. We use initial phage panning rounds performed with human phage display libraries with a total complexity of 10^{10} transformants and monovalent display to enrich high-affinity binders with binding affinities in the low-nanomolar range and to take advantage of the flexibility of the phage panning procedure. Following the initial enrichment of binders, the entire diversity is batch-recloned in the final drug format, e.g. full-length IgG or novel bispecific antibodies, into the AbSieve Display system and sorted for best expression and affinities in FACS. The proof-reading mechanism of the yeast cell excludes misfolded antibodies, and the system favors the selection of stable molecules. Switching from display to secretion mode without recloning step allows direct screening of antibodies in yeast culture supernatants and fast purification for detailed characterization. We compared antibodies identified by scFv Phage Display and AbSieve IgG Display and demonstrate the power and applicability of the two display methodologies for the identification of better drug candidates.

Human scFv Phage Display libraries

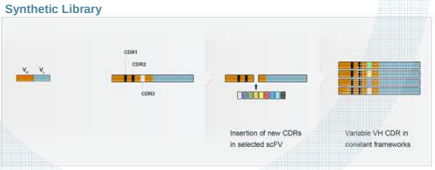
Three different scFv Phage Display libraries with a total complexity of 10^{10} were used as starting material for the selections:

- Natural library: cloned from IgM derived antibody sequences from human donors
- Synthetic library: created by new VH CDR3 sequences into defined scFv
- Semisynthetic library : VH is of synthetic, VL of natural origin.

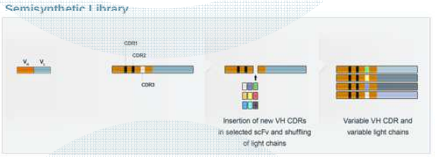
The libraries have been subjected to either three panning rounds or recloned into the AbSieve IgG Display vectors after the second panning round.

Natural Library

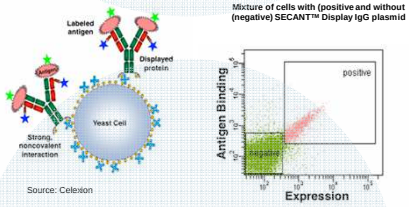
Synthetic Library



Semisynthetic Library



Yeast Display of full-length IgG



Phage scFv pools after two panning rounds are used to build up target specific IgG libraries displayed on the surface of yeast cells.

For full-length IgG display the SECANT™ platform (proprietary technology of Celexion LLC) was used.

The IgG is expressed as a fusion with a biotin acceptor peptide. A co-expressed biotin ligase attaches biotin to the BAP tag on the expressed protein. Avidin is attached to the outer wall of the host cell, and the biotinylated IgG is captured on the surface upon secretion.

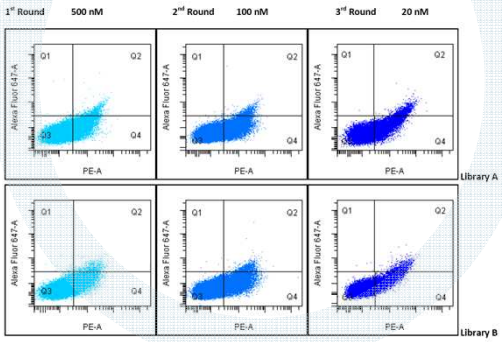
Double staining of cell populations with antibodies against a tag sequence in the displayed IgG and with labeled antigen is used to plot the cells according to antigen binding and IgG expression and to sort the part of the population with best expression and antigen binding level.

After two or three sorting rounds the plots showed good enrichments of binders in the population.

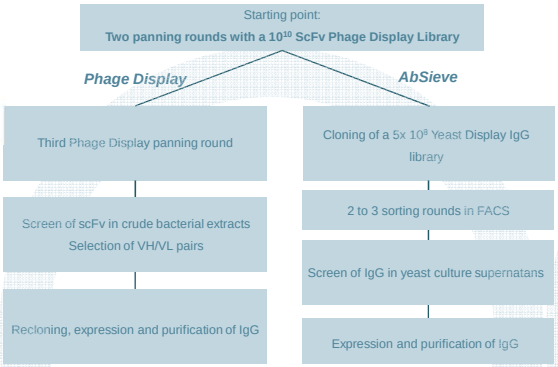
Fluorescence assisted cell sorting (FACS)

Yeast cells were stained with anti FLAG antibody to detect the IgG molecules displayed on the surface of the cells and with His-tagged antigen and the respective fluorescently labeled secondary antibody.

FACS plots of stained cell populations allow calculation of IgG displaying and antigen binding cells. In this actual case we started with 20 to 40% IgG displaying and <5% antigen binding cells. The rate of expressing cells increased to 50 to 70% after the first sorting round. After two or three sorting rounds enrichment of binders was indicated by the FACS plots. Screens of supernatants of single clones in ELISA confirmed enrichments of binders, reaching hit rates of >80%.



Workflow: Phage Display and AbSieve

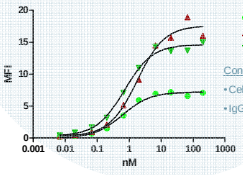


Affinity of selected IgG

The affinity of selected IgG was probed in Biacore and in titrations on the surface of transfected antigen expressing HEK293 cells.

- 2 of 9 tested IgG with VH/VL pairs selected in Phage Display and 12 of 14 IgG with unrelated VH CDR3 showed binding signals in Biacore.
- All measured affinities of Phage derived IgG and AbSieve IgG ranged between 0.5 and 7 nM

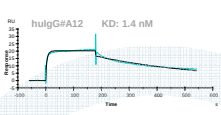
Titration of purified IgG on the surface of transfected cells



Conditions for cell surface titrations:

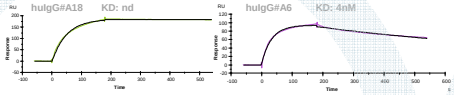
- Cells were stained for 45 min at 4°C
- IgG detected with anti FLAG antibody

SPR affinity measurements with 0.1 µM target Fc fusion protein



Biacore X100 measurements:

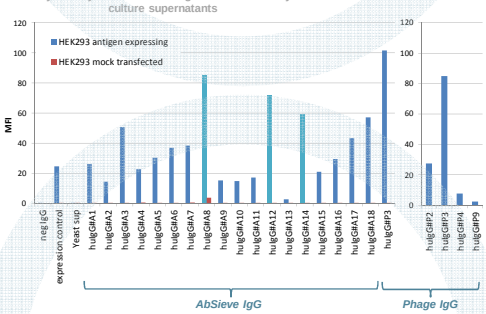
- purified IgG captured with anti human Fab antibody on CM5 sensor chip
- target-Fc fusion protein as soluble analyte
- 180 sec contact; 360 sec dissociation time
- flow rate of 25 µl/min



Cell surface binding

The binding of selected IgG to the antigen expressed on the surface of transfected HEK293 cells was tested in flow cytometry. Cells were stained for 45 min at 4°C, and IgG were detected with anti FLAG antibody. Three VL variants with identical VH sequence are marked in lighter blue.

Flow cytometry test of selected IgG measured with yeast culture supernatants



Sequence Diversity in selected scFv and IgG pools

Sets of target-specific scFv and IgG after 2nd and 3rd panning/sorting round were sequenced.

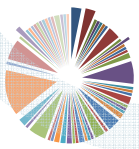
The frequencies of 60 unrelated sequence families found in the target-specific scFv pool from Phage Display are plotted in the pie chart. Only four of these, indicated by shifted segments, occurred again among the 17 unrelated sequence families selected from the AbSieve IgG pool.

The selected IgG pool contained sets of VL variants for several heavy chains which is also obvious from HRM profiles of VH and VL sequences.

Phage scFv Display

- 60 unrelated VH CDR3 in 128 sequences
- 4 VH and 7 VL germline families represented
- 4 VH CDR3 correspond to sequences in IgG pool. These sequences are indicated by shifted segments in the pie chart.

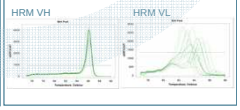
Frequencies of sequence families:



AbSieve IgG Display

- 17 unrelated VH CDR3 in 287 screened colonies
- 3 VH and 4 VL germline families represented
- Some heavy chains occurred in combination with several VL chains

DNA High resolution melting (HRM) profiles revealed higher diversity in VL compared to VH chains:



Diverse pools of target specific antibodies including sequences derived from different families of germline genes can be obtained with both display technologies, but we found only minor overlap between antibody sequences isolated by either approach.

Highly stringent sorting conditions reduced the VH diversity in AbSieve IgG Yeast Display whereas more diversity was retained during scFv Phage Panning.

We suppose significant selection pressure acting on antigen binding affinity to be the major driving force during our FACS sorting process.

Summary

Diverse pools of target-specific antibody sequences were isolated with Phage Display and the novel combination of Phage and Yeast Display (AbSieve). Surprisingly, different antibody sequences were isolated by the one or the other technology, for example the most frequent VH sequences isolated from Phage Display didn't show up in the AbSieve approach. Tested IgG with VH/VL pairs selected in Phage Display were produced and purified with the same yields as IgG from AbSieve, and only a minor percentage of VH/VL pairs in the selected scFv pool is supposed to be problematic for IgG expression. However, the AbSieve selected pool of antibody sequences was reduced in VH sequence diversity compared to the outcome after three Phage panning rounds, whereas some of the selected VH chains occurred in combination with several light chains after AbSieve but not in the Phage selected pools. These findings reflect the interplay of two unique features in the AbSieve process: The built-in light chain shuffling step during IgG library cloning ensures a more complete representation of VL pairings for a given heavy chain than the initial scFv Phage Display library, and the FACS sorting process is more stringent and more effective than Phage Display in deselecting weak binders. Therefore the AbSieve approach is regarded as valuable tool for efficient sorting of VH/VL pairs with best performance in IgG or other antibody format.